



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: PCT/NL99/00417  (22) International Filing Date: 2 July 1999 (02.07.99)  (30) Priority Data: 98202233.7 2 July 1998 (02.07.98) EP  (71) Applicant (for all designated States except US): STICHTING SKELETAL TISSUE ENGINEERING GROUP AMSTER- DAM [NL/NL]; c/o Academisch Ziekenhuis Vrije Univer- siteit, De Boelelaan 1117, NL-1081 HV Amsterdam (NL).  (72) Inventors; and (75) Inventors/Applicants (for US only): BURGER, Elisabeth, Henriëtte [NL/NL]; Korteraarsweg 107, NL-2461 GK Ter Aar (NL); VAN NIEUW AMERONGEN, Arie [NL/NL]; G. van Nijenroodstraat 136, NL-3621 GK Breukelen (NL); WUJSMAN, Paulus, Ignatius, Jozef, Maria [NL/NL]; Lupine Oord 29, NL-3991 VG Houten (NL).  (74) Agent: VAN SOMEREN, Petronella, Francisca, Hendrika, Maria; Arnold &amp; Siedsma, Sweelinkplein 1, NL-2517 GK The Hague (NL).</p>		<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments. In English translation (filed in Dutch).</i></p>
<p>(54) Title: BONE CEMENT WITH ANTIMICROBIAL PEPTIDES</p> <p>(57) Abstract</p> <p>The invention relates to bone material for the prevention and treatment of osteomyelitis, which material is provided with antimicrobial peptides (AMPs) consisting of an amino acid chain which contains a domain of 10 to 25 amino acids, wherein the majority of the amino acids of the one half of the domain are positively charged amino acids and the majority of the amino acids of the other half of the domain are uncharged amino acids, which AMPs can be released to the surrounding area for a period of time and wherein the bone material forms bone cement after curing and the AMPs are distributed homogeneously in the cured bone cement. The invention further relates to a method of manufacturing the bone material, wherein the bone material is cured to bone cement and wherein the AMPs are distributed homogeneously in the cured bone cement.</p>		

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**BONE CEMENT WITH ANTIMICROBIAL PEPTIDES**

The invention relates to the use of antimicrobial peptides (AMP) in calcium phosphate bone cement and forms a system which provides for slow release of the AMP for prevention and treatment of infections of the bone  
5 (osteomyelitis) and the surrounding soft tissues.

Preventing infections of the soft tissues and the bone after operations remains a cause for concern in orthopaedic and trauma surgery. Infection of bone tissues (osteomyelitis) and/or the surrounding soft tissue is  
10 very difficult to cure and this is a reason why stringent prevention is required. At this moment granules of polymethyl methacrylate (PMMA-granules) are used for this purpose. When they are placed in the surgical wound they function as a slow release system for obtaining high  
15 local concentrations of antibiotics, while the systemic concentrations remain low. Such granules are however non-re-absorbable and an additional operation is therefore necessary. The intensive use of antibiotics in human and veterinary medicine has further resulted in large scale  
20 resistance of bacteria and fungi to antibiotics such as gentamicin. New therapies for prevention and treatment of for instance osteomyelitis are therefore urgently required.

The present invention provides for this purpose a  
25 new system for the prevention and treatment of osteomyelitis, which makes use of a re-absorbable calcium phosphate cement carrier and a new class of antibiotic agents, the so-called antimicrobial peptides (AMPs).

The AMPs used in the invention are peptides  
30 consisting of an amino acid chain which contains a domain of 10 to 25 amino acids, wherein the majority of the amino acids of the one half of the domain are positively

charged amino acids and the majority of the other half of the domain are uncharged amino acids.

- The structure of these peptides has a number of variations. Firstly, the domain can form an  $\alpha$ -helix, of which at least a majority of the positions 1, 2, 5, 6, 9 (12, 13, 16, 19, 20, 23 and 24) contains a positively charged amino acid, position 8 is a positive or an uncharged amino acid and at least a majority of the positions 3, 4, 7, 10, (11, 14, 15, 17, 18, 21, 22, 25) contains an uncharged amino acid. These peptides have a lateral amphipathicity, i.e. a maximum hydrophobic moment at 100°. Stated simply, these peptides are hydrophobic on the left side and hydrophilic on the right side or vice versa. These peptides are referred to herein as "type I".
- 15 The domain can further form an  $\alpha$ -helix, of which at least a majority of the positions 1, 2, 5, 6, 9 (12, 13, 16, 19, 20, 23 and 24) contains an uncharged amino acid, position 8 is a positive or an uncharged amino acid and at least a majority of the positions 3, 4, 7, 10, (11, 20 14, 15, 17, 18, 21, 22, 25) contains a positively charged amino acid. These peptides have a lateral amphipathicity, i.e. a maximum hydrophobic moment at 100°. Stated simply, these peptides are hydrophobic on the right side and hydrophilic on the left side or vice versa. These
- 25 peptides are designated "type II" herein and are in principle mirror-symmetrical to type I peptides.

- In addition, the domain can form an  $\alpha$ -helix, wherein at least a majority of the positions 1 to 6 (or 7 or 8 or 9 or 10 or 11 or 12) contains an uncharged amino acid and 30 a positively charged amino acid is found at position 7 (or 8 or 9 or 10 or 11 or 12 or 13) to 25. These peptides have a longitudinal amphipathicity, i.e. a minimum hydrophobic moment at 100°. These peptides are hydrophobic on their "top" and hydrophilic on their "bottom". Such
- 35 peptides are designated "type III".

- Conversely, the domain can form an  $\alpha$ -helix, wherein at least a majority of the positions 1 to 6 (or 7 or 8 or 9 or 10 or 11 or 12) contains a positively charged amino acid and an uncharged amino acid is found at position 7 (or 8 or 9 or 10 or 11 or 12 or 13) to 25. These peptides likewise have a longitudinal amphipathicity and therefore a minimum hydrophobic moment at  $100^\circ$ . These peptides are hydrophobic on their "bottom" and hydrophilic on their "top". Such peptides are designated "type IV".
- 10 Finally, the domain can form a so-called  $\beta$ -strand and contain a positively charged amino acid on at least a majority of the positions 1, 3, 5, 7, 9 (11, 13, 15, 17, 19, 21, 23 and 25) and an uncharged amino acid on at least a majority of the positions 2, 4, 6, 8, 10, (12, 15 14, 16, 18, 20, 22, 24). Such a  $\beta$ -strand is laterally amphipathic and has a maximum hydrophobic moment at  $180^\circ$ . The  $\beta$ -strand structure is flatter than the  $\alpha$ -helix and, stated simply, is hydrophobic on the left and hydrophilic on the right or vice versa. These are "type V" peptides.
- 20 The positively charged amino acids are preferably chosen from the group consisting of ornithine (O), lysine (K), arginine (R) and histidine (H), while the uncharged amino acids are preferably chosen from the group consisting of the aliphatic amino acids glycine (G), 25 alanine (A), valine (V), leucine (L), isoleucine (I), the amino acids with a dipolar side chain methionine (M), asparagine (N), glutamine (Q), serine (S), threonine (T), the amino acids with an aromatic side chain phenylalanine (F), tyrosine (Y), tryptophan (W). Amino acids on the 30 border between hydrophilic and hydrophobic can be chosen from both groups or from the remaining amino acids.
- Hardly any difference in activity can in principle be detected when one of the positive amino acids and/or one of the uncharged amino acids is replaced by a random 35 amino acid. The majority of the positively charged amino acids is therefore preferably the total number of

positively charged amino acids minus 1 and the majority of the uncharged amino acids is preferably the total number of uncharged amino acids minus 1.

The domain can be a part of a larger peptide but can itself also make up the entire peptide. When the domain forms part of a larger peptide, the C-terminal and/or N-terminal amino acids which are then additionally present can be random amino acids.

The following peptides of the type I are particularly recommended:

	KRLFKEKLFSLRKY	(peptide 3)
	KRLFKELLFSLRKY	(peptide 4)
	KRLFKEKKSLRKY	(peptide 5)
	KRLFKELLKSLRKY	(peptide 6)
15	OOLFOELOOSLOOY	(peptide 7)
	OOLFOELLOSLOOY	(peptide 8)
	KRLFKKLKFSLRKY	(peptide 9)
	KRLFKKLLFSLRKY	(peptide 10)

A preferred peptide of the type III has the following amino acid sequence:

LLLFLKKRKKRKY (peptide 11)

The peptides according to the invention can also contain further modifications. These modifications are for instance an N-terminal amide ring, for instance with acetic acid anhydride, or an alternative cleavage of the synthesis resin by which the C-terminus is modified. For this latter a replacement of the C-terminal carboxylic acid group by an amide, ester, ketone, aldehyde or alcohol group can be envisaged. Peptides with such a modification are for instance:

KRLFKEKLFSLRKY-amide (peptide 12)

KRLFKELLFSLRKY-amide (peptide 13)

In addition to single peptides, oligomers can also be made. These are preferably linear oligomers of the peptides according to the invention. The coupling can be head-to-head and tail-to-tail as well as head-to-tail,

- either by direct synthesis or by post-synthetic enzymatic coupling. For a trans-membrane pore formation a minimum peptide length is required. Oligomers of the peptides according to the invention are double length and thereby
- 15 better able in principle to span the whole phospholipid double layer of the bacterial cell membrane at one time. The activity of the peptide could hereby improve even further. In addition, extension of the peptides provides stabilisation of the helix conformation. A spacer must
- 20 usually be inserted. In direct synthesis of head-to-tail coupled oligomers a spacer can be inserted to size by the use of a chain of unnatural amino acids of the correct length, for instance  $\beta$ -alanine,  $\gamma$ -amino butyric acid,  $\epsilon$ -amino caproic acid, etc. Heterodifunctional coupling
- 25 reagents, such as are commercially available for coupling peptide antigens to carrier proteins (for instance 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC), *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS), *N*-succinimidyl 3-[pyridyldithio]propionate (SPDD) etc.) are
- 30 used to make linear oligomers with an inserted spacer. For head-to-head and tail-to-tail couplings can be used trivalent amino acids such as asparagine acid (D), glutamine acid (E), ornithine (O), lysine (K), serine (S), cysteine. Such oligomers are for instance:
- 25   KKKFHEKHHSHRGYC-CYGRHSHHKEHFRRK           (peptide 14)  
      YGRHSHHKEHFRRKC-CKRKFHEKHHSHRGY           (peptide 15)  
       $^{\alpha}\text{N}, ^{\alpha}\text{N}-(\text{KKKFHEKHHSHRGY})_2\text{K-amide}$            (peptide 16)  
       $^{\alpha}\text{N}, ^{\alpha}\text{N}-(\text{KRLFKEKLFSLRKY})_2\text{K-amide}$            (peptide 17)  
       $^{\alpha}\text{N}, ^{\alpha}\text{N}-(\text{KRLFKKLFSLRKY})_2\text{K-amide}$            (peptide 18)
- 30 Peptides 14 and 15 are obtained by synthesis of peptide 2 with an additional C-terminal respectively N-terminal cysteine, whereafter the oligomer is obtained by air oxidation. Peptides 16, 17 and 18 are obtained by making use of the Multiple Antigenic Peptide (MAP) strategy,
- 35 wherein a lysine having on both the  $\alpha$ - and on the  $\epsilon$ -amino group an Fmoc protection was used as first amino acid on

the synthesis resin, whereby two identical amino acid chains (peptides 2, 3 and 9) were synthesized simultaneously on one lysine molecule.

The peptides described herein have no or hardly any haemolytic activity in physiological buffers such as PBS (phosphate-buffered saline solution). A low activity against erythrocytes of human origin is an indication of low toxicity. This selectivity is essential for the use of these peptides as antibiotics.

The peptides have a wide spectrum of antibacterial and antifungal activity, even against methycillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa (which is particularly dangerous in the case of osteomyelitis) and amphotericin-B-resistant Candida albicans.

The invention further makes use of bone material which after curing forms bone cement and wherein the AMPs are distributed homogeneously in the cured bone cement. It is biocompatible, re-absorbable and inert, and forms at body temperature. The final cement moreover has sufficient strength and stiffness to serve as bone replacement.

It has been found according to the invention that the inclusion of the AMPs in the cement does not affect the mechanical properties thereof.

In order to include the AMPs in the cement, they are dissolved in a liquid medium, preferably water, and mixed with the bone material before or after curing thereof.

A blood protein-containing solution, in particular albumin, is preferably used to hold the AMPs in solution, in order to ensure a homogeneous distribution of the AMPs in the final cured bone cement.

In a preferred embodiment bone material contains calcium phosphate. With a view to the biocompatibility this is particularly a mixture of dicalcium phosphate,



tricalcium phosphate, tetracalcium phosphate and/or hydroxyl-apatite.

The invention further relates to a method of manufacturing a bone material according to the invention, 5 wherein the bone material is cured to bone cement and wherein the AMPs are distributed homogeneously in the cured bone cement. As stated, the AMPs are dissolved in a liquid medium, preferably water, and mixed with the bone material before or after curing thereof. The AMPs are 10 preferably mixed with the bone material after curing. A longer release period is thus provided in which the AMPs can be released to the surrounding area after arranging of the bone material. The starting point here in each case is that the AMPs are always active only where this 15 is necessary.

The invention also relates to a device for administering bone material provided with AMPs according to the invention, wherein provision is made for at least two compartments for separately containing the bone 20 material and AMPs, a mixing chamber for mixing the bone material and the AMPs and a spray nozzle for spraying the mixture out of the mixing chamber.

The invention will be further elucidated with reference to a discussion of a number of tests in 25 accordance with preferred variants of the invention, wherein the procedures for manufacturing the present bone material with added AMPs will be discussed.

1. A sterile cement powder consists of a mixture of 30 alpha-tricalcium phosphate, tetracalcium phosphate-monoxide en dicalcium phosphate dibasic in a ratio of 75:20:5, or otherwise if desired.
2. A sterile AMP solution (solution (A)) consists of 4 35 mM HCl in water having dissolved therein 0.1% beef

or human serum albumin and AMPs in a concentration as required varying from  $2 \times 10^{-5}\%$  to 2%.

3. A second sterile solution (solution (B)) consists of  
5 water having dissolved therein 12% sodium succinate and 5% chondroitin succinate.
4. Solution (A) is mixed 1 to 1 with solution (B) under  
sterile conditions.
- 10 5. One volume part solution (A+B) is mixed with two volume parts cement powder under sterile conditions. This can take place:
  - 15 a. in a dish and mixed with a spatula, whereafter the cement paste is arranged immediately in-situ in the body of the patient and there cures;
  - 20 b. via a spray with two chambers, one of which contains the cement powder and the other solution (A+B); using the spray, powder and liquid are brought together in-situ in the body, whereafter the mixture cures at this  
25 location.
  - c. in a dish, mould or container, whereafter the mixture cures outside the body and is optionally ground to a powder of the desired  
30 granule size, whereafter it is arranged in the body of the patient.
6. One volume part solution B is mixed with two  
35 volume parts cement powder under sterile conditions in a dish, mould or container, whereafter the mixture cures and is ground to a

powder of the desired granule size. The cured cement is then incubated for 1 or more hours in solution A, whereafter the cement with absorbed AMFs is dried and stored in dry form until it is arranged in the body of the patient.

5

**CLAIMS**

1. Bone material for the prevention and treatment of osteomyelitis, which material is provided with antimicrobial peptides (AMPs) consisting of an amino acid chain which contains a domain of 10 to 25 amino acids,  
5 wherein the majority of the amino acids of the one half of the domain are positively charged amino acids and the majority of the amino acids of the other half of the domain are uncharged amino acids, which AMPs can be released to the surrounding area for a period of time and  
10 wherein the bone material forms bone cement after curing and the AMPs are distributed homogeneously in the cured bone cement.

2. Bone material as claimed in claim 1,  
**characterized in that** the domain forms an  $\alpha$ -helix and at  
15 least at a majority of the positions 1, 2, 5, 6, 9 (12, 13, 16, 19, 20, 23 and 24) contains a positively charged amino acid, at position 8 a positive or an uncharged amino acid and at least at a majority of the positions 3,  
4, 7, 10, (11, 14, 15, 17, 18, 21, 22, 25) contains an  
20 uncharged amino acid.

3. Bone material as claimed in claim 2,  
**characterized in that** the positively charged amino acids are chosen from the group consisting of ornithine (O), lysine (K), arginine (R) and histidine (H).

25 4. Bone material as claimed in claim 2 or 3,  
**characterized in that** the uncharged amino acids are chosen from the group consisting of the aliphatic amino acids glycine (G), alanine (A), valine (V), leucine (L), isoleucine (I), the amino acids with a dipolar side chain  
30 methionine (M), asparagine (N), glutamine (Q), serine (S), threonine (T), the amino acids with an aromatic side chain phenylalanine (F), tyrosine (Y), tryptophan (W).

5. Bone material as claimed in claims 2-4,  
**characterized in that** the majority of the positively charged amino acids is the total number of positively charged amino acids minus 1.
- 5 6. Bone material as claimed in claims 2-5,  
**characterized in that** the majority of the uncharged amino acids is the total number of uncharged amino acids minus 1.
7. Bone material as claimed in claims 2-6,  
10 **characterized in that** the domain makes up the entire peptide.
8. Bone material as claimed in claims 2-7, of which the domain has the following amino acid sequence:  
KRLFKELKFSLRKY (peptide 3).
- 15 9. Bone material as claimed in claims 2-7, of which the domain has the following amino acid sequence:  
KRLFKELLFSLRKY (peptide 4).
10. Bone material as claimed in claims 2-7, of which the domain has the following amino acid sequence:  
20 KRLFKELKKSLRKY (peptide 5).
11. Bone material as claimed in claims 2-7, of which the domain has the following amino acid sequence:  
KRLFKELLKSLRKY (peptide 6).
12. Bone material as claimed in claims 2-7, of which  
25 the domain has the following amino acid sequence:  
OOLFOELOOSLOOY (peptide 7).
13. Bone material as claimed in claims 2-7, of which the domain has the following amino acid sequence:  
OOLFOELLOSLOOY (peptide 8).
- 30 14. Bone material as claimed in claims 2-7, of which the domain has the following amino acid sequence:  
KRLFKKLKFSLRKY (peptide 9).
15. Bone material as claimed in claims 2-7, of which the domain has the following amino acid sequence:  
35 KRLFKLLFSLRKY (peptide 10).

16. Bone material as claimed in claim 1,  
**characterized in that** the domain forms an  $\alpha$ -helix and at  
least at a majority of the positions 1 to 6 (or 7 or 8 or  
9 or 10 or 11 or 12) contains an uncharged amino acid and  
5 at position 7 (or 8 or 9 or 10 or 11 or 12 or 13) to 25 a  
positively charged amino acid.

17. Bone material as claimed in claim 1,  
**characterized in that** the domain forms an  $\alpha$ -helix and at  
least at a majority of the positions 1 to 6 (or 7 or 8 or  
10 9 or 10 or 11 or 12) contains a positively charged amino  
acid and at position 7 (or 8 or 9 or 10 or 11 or 12 or  
13) to 25 an uncharged amino acid.

18. Bone material as claimed in claim 16 or 17,  
**characterized in that** the positively charged amino acids  
15 are chosen from the group consisting of ornithine (O),  
lysine (K), arginine (R) and histidine (H).

19. Bone material as claimed in claim 16, 17 or 18,  
**characterized in that** the uncharged amino acids are  
chosen from the group consisting of the aliphatic amino  
20 acids glycine (G), alanine (A), valine (V), leucine (L),  
isoleucine (I), the amino acids with a dipolar side chain  
methionine (M), asparagine (N), glutamine (Q), serine  
(S), threonine (T), the amino acids with an aromatic side  
chain phenylalanine (F), tyrosine (Y), tryptophan (W).

25 20. Bone material as claimed in claims 16-19,  
**characterized in that** the majority of the positively  
charged amino acids is the total number of positively  
charged amino acids minus 1.

21. Bone material as claimed in claims 16-20,  
30 **characterized in that** the majority of the uncharged amino  
acids is the total number of uncharged amino acids minus  
1.

22. Bone material as claimed in claims 16-21,  
**characterized in that** the domain makes up the entire  
35 peptide.

23. Bone material as claimed in claims 16 and 18-22, of which the domain has the following amino acid sequence:

LLLFLKKRKKRKY (peptide 11).

5 24. Bone material as claimed in claim 1, **characterized in that** the domain forms a so-called  $\beta$ -strand and contains a positively charged amino acid on at least a majority of the positions 1, 3, 5, 7, 9 (11, 13, 15, 17, 19, 21, 23 and 25) and an uncharged amino acid on 10 at least a majority of the positions 2, 4, 6, 8, 10, (12, 14, 16, 18, 20, 22, 24).

25. Bone material as claimed in claim 24, **characterized in that** the positively charged amino acids are chosen from the group consisting of ornithine (O), 15 lysine (K), arginine (R) and histidine (H).

26. Bone material as claimed in claim 24, **characterized in that** the uncharged amino acids are chosen from the group consisting of the aliphatic amino acids glycine (G), alanine (A), valine (V), leucine (L), 20 isoleucine (I), the amino acids with a dipolar side chain methionine (M), asparagine (N), glutamine (Q), serine (S), threonine (T), the amino acids with an aromatic side chain phenylalanine (F), tyrosine (Y), tryptophan (W).

27. Bone material as claimed in claims 24-26, 25 **characterized in that** the majority of the positively charged amino acids is the total number of positively charged amino acids minus 1.

28. Bone material as claimed in claims 24-27, **characterized in that** the majority of the uncharged amino 30 acids is the total number of uncharged amino acids minus 1.

29. Bone material as claimed in claims 24-28, **characterized in that** the domain makes up the entire peptide.

30. Bone material as claimed in claims 1-29, wherein the N-terminus is amidated.

31. Bone material as claimed in claims 1-30, wherein the C-terminal carboxylic acid group is replaced by an  
5 amide, ester, ketone, aldehyde or alcohol group.

32. Method of manufacturing bone material as claimed in any of the claims 1-31, wherein the bone material is cured to bone cement and wherein the AMPs are distributed homogeneously in the cured bone cement.

10 33. Method as claimed in claim 32, wherein the AMPs are dissolved in liquid medium, preferably water, and mixed with the bone material after curing thereof.

34. Method as claimed in claim 32 or 33, wherein the cured bone cement is formed to a granulate.

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## INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/NL 99/00417

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61L24/10 A61L27/22 A61K38/10 A61K38/17		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 A61L		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39202 A (OSTEOGENICS INC) 12 December 1996 (1996-12-12) page 44, line 4; claims	1, 32-34
A	EP 0 510 912 A (MORINAGA MILK INDUSTRY CO LTD) 28 October 1992 (1992-10-28) claims; examples	1-34
A	WO 97 18827 A (INTRABIOTICS PHARMACEUTICALS I) 29 May 1997 (1997-05-29) claims; examples	1
A	WO 94 15653 A (GENENTECH INC) 21 July 1994 (1994-07-21) claims	1
-/-		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
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Date of the actual completion of the international search		Date of mailing of the international search report
2 November 1999		16/11/1999
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  ESPINOSA, M

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International Application No  
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 20064 A (AMERICAN DENTAL ASS) 15 September 1994 (1994-09-15) claims; examples 1-10 -----	1,32-34
A	DUCAN YU ET AL: "SELF-SETTING HYDROXYAPATITE CEMENT: A NOVEL SKELETAL DRUG-DELIVERY SYSTEM FOR ANTIBIOTICS" JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 81, no. 6, 1 June 1992 (1992-06-01), pages 529-531, XP000271282 ISSN: 0022-3549 -----	1,32-34
A	WO 92 01462 A (SCRIPPS RESEARCH INST) 6 February 1992 (1992-02-06) claims -----	1
E	WO 99 37678 A (HELMERHORST EVA JOSEPHINE ;NIEUW AMERONGEN ARIE VAN (NL); STICHTIN) 29 July 1999 (1999-07-29) the whole document -----	1-34

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/NL 99/00417

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9639202 A	12-12-1996	AU 6149696 A	24-12-1996
		BR 9608344 A	05-01-1999
		CA 2223596 A	12-12-1996
		EP 0830149 A	25-03-1998
		JP 11506659 T	15-06-1999
EP 0510912 A	28-10-1992	AU 664697 B	30-11-1995
		AU 1514692 A	29-10-1992
		CA 2066997 A	25-10-1992
		DE 69223844 D	12-02-1998
		DE 69223844 T	16-04-1998
		DK 510912 T	09-02-1998
		JP 5148295 A	15-06-1993
		NZ 242437 A	27-07-1993
		US 5424396 A	13-06-1995
WO 9718827 A	29-05-1997	AU 704851 B	06-05-1999
		AU 1162997 A	11-06-1997
		AU 7739496 A	11-06-1997
		CA 2238429 A	29-05-1997
		CZ 9801591 A	14-10-1998
		CZ 9801592 A	16-12-1998
		EP 0862448 A	09-09-1998
		EP 0865292 A	23-09-1998
		HU 9901183 A	28-07-1999
		NO 982310 A	22-07-1998
		NO 982311 A	22-07-1998
		PL 326924 A	09-11-1998
		WO 9718826 A	29-05-1997
WO 9415653 A	21-07-1994	AT 153535 T	15-06-1997
		AU 671721 B	05-09-1996
		AU 6026294 A	15-08-1994
		CA 2151486 A	21-07-1994
		DE 69403439 D	03-07-1997
		DE 69403439 T	23-10-1997
		DK 679097 T	22-12-1997
		EP 0679097 A	02-11-1995
		ES 2105641 T	16-10-1997
		GR 3024277 T	31-10-1997
		JP 8505548 T	18-06-1996
		US 5422340 A	06-06-1995
WO 9420064 A	15-09-1994	US 5522893 A	04-06-1996
		AT 183382 T	15-09-1999
		AU 684722 B	08-01-1998
		AU 4923993 A	26-09-1994
		BR 9307825 A	14-11-1995
		CA 2157890 A, C	15-09-1994
		DE 69326082 D	23-09-1999
		EP 0688202 A	27-12-1995
		JP 8510713 T	12-11-1996
		US 5542973 A	06-08-1996
		US 5545254 A	13-08-1996
		US 5695729 A	09-12-1997
WO 9201462 A	06-02-1992	CA 2047317 A	20-01-1992
		JP 6504260 T	19-05-1994

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/NL 99/00417

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9201462 A		US 5294605 A	15-03-1994
WO 9937678 A	29-07-1999	NONE	